Available online at www.sciencedirect.com

ScienceDirect

Virology 359 (2007) 82–91

VIROLOGY

www.elsevier.com/locate/yviro

Paramyxovirus Sendai virus V protein counteracts innate virus clearance through IRF-3 activation, but not via interferon, in mice

Katsuhiro Kiyotani [a], Takemasa Sakaguchi [a], Atsushi Kato [b], Yoshiyuki Nagai [c], Tetsuya Yoshida [a,*]

[a] *Department of Virology, Graduate School of Biomedical Sciences, Hiroshima University, 1-2-3 Kasumi, Minami-ku, Hiroshima 734-8551, Japan*
[b] *Department of Virology 3, National Institute of Infectious Diseases, Musashi-Murayama, Tokyo 208-0011, Japan*
[c] *Center of Research Network for Infectious Diseases, RIKEN, Tokyo 100-0006, Japan*

Received 29 June 2006; returned to author for revision 7 August 2006; accepted 29 August 2006
Available online 6 October 2006

Abstract

The present study was undertaken to clarify the role of Sendai virus (SeV) V protein, which has been shown to downregulate IFN-β induction through inhibition of IRF-3 activation, in viral pathogenesis. Mice infected with rSeV mutants, deficient in V expression or expressing V lacking the C-terminus, had several-fold higher IFN activity levels in the lungs than those in wild-type virus-infected mice, and the mutant viruses were rapidly excluded from the lung from the early phase of infection before induction of acquired immunity. In addition, the unique early clearance of the mutants did not occur in IRF-3 knockout (KO) mice. However, high titers of IFN were detected even in the infected KO mice. Furthermore, early clearance of the mutant viruses was also observed in IFN signaling-deficient mice, IFN-α/β receptor KO mice and STAT1 KO mice. These results indicate that SeV V protein counteracts IRF-3-mediated innate antiviral immunity for efficient virus replication and pathogenesis in mice, but it is not IFN.

Keywords: Sendai virus; Pathogenesis; V protein; IRF-3; Interferon

Introduction

The subfamily *Paramyxovirinae* of the family *Paramyxoviridae* includes many human and animal pathogens such as measles virus as well as the newly emergent Hendra and Nipah viruses, which can cause fatal zoonotic infections (Lamb and Kolakofsky, 2001). Viruses are subjected to various antiviral host responses upon infection, and interferon (IFN) responses play important roles in early innate immunity and in modulation of subsequent acquired immunity (Goodbourn et al., 2000; Sen, 2001; Taniguchi and Takaoka, 2002). Recent extensive studies have revealed that most paramyxoviruses encode specific viral proteins, accessory V and/or C proteins encoded by the *P* gene to circumvent the IFN system by blocking IFN signaling and limiting the production of IFN in infected cells (Garcia-Sastre, 2001, 2004; Goodbourn et al., 2000; Gotoh et al., 2001, 2002; Horvath, 2004; Nagai and Kato, 2004). For instance, in simian virus 5 (SV5), which belongs to the genus *Rubulavirus* of *Paramyxovirinae,* the V protein targets STAT1 for proteasome-mediated degradation, thereby blocking both IFN-α/β and IFN-γ signaling within infected cells (Didcock et al., 1999a, 1999b), while the C protein, instead of the V protein, counteracts IFN signaling in the case of Sendai virus (SeV) of the genus *Respirovirus* of *Paramyxovirinae* (Didcock et al., 1999a, 1999b; Garcin et al., 1999; Gotoh et al., 1999). SeV C protein blocks IFN signaling by inducing abnormal phosphorylation and dephosphorylation of STAT1 and STAT2 and its binding to STAT1 (Gotoh et al., 2003a, 2003b; Kato et al., 2004; Komatsu et al., 2000, 2002; Takeuchi et al., 2001). In addition, it has become evident that V and C proteins downregulate the production of IFN in infected cells as well. The V proteins of SV5 and SeV have been shown to inhibit induction of IFN-β through blocking interferon regulatory factor (IRF)-3 and NF-κB activation (He et al., 2002; Komatsu et al., 2004; Poole et al., 2002). SeV C protein also blocks the signaling pathway leading

* Corresponding author. Fax: +81 82 257 5159.
E-mail address: tetsuyay@hiroshima-u.ac.jp (T. Yoshida).

0042-6822/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.virol.2006.08.053

to IRF-3 activation (Komatsu et al., 2004). Furthermore, RNA helicases, retinoic acid inducible gene I (*RIG-I*) and melanoma differentiation-associated gene 5 (*mda-5*) products, have recently been identified as the host factors for detecting intracellular dsRNA produced by virus replication and initiating antiviral responses (Andrejeva et al., 2004; Yoneyama et al., 2004). In addition, the cysteine-rich C-terminal domain of V protein, highly conserved among paramyxoviruses, has been demonstrated to bind mda-5, resulting in inhibition of activation of the IFN-β promoter through IRF-3 (Andrejeva et al., 2004; Yoneyama et al., 2005). Thus, the V and C proteins of paramyxoviruses are thought to play essential roles for evading host innate immunity, especially the IFN system, upon virus infection. However, it is still unclear how the inhibitory capacity of V protein against IRF-3 activation revealed *in vitro* is actually involved in *in vivo* viral replication and pathogenesis.

SeV infects exclusively respiratory epithelial cells of rodents and causes fatal bronchopneumonia (Ishida and Homma, 1978), and experimental infection of mice with SeV has provided a useful model for investigation of viral pathogenesis (Fujii et al., 2002; Kato et al., 1997a, 1997b; Kiyotani et al., 1990; Kurotani et al., 1998; Sakaguchi et al., 2003; Tashiro and Homma, 1983). SeV is an enveloped virus possessing a non-segmented single-stranded negative-sense RNA genome containing six genes in the order 3′-(leader)-*N-P-M-F-HN-L*-(trailer)-5′. The accessory proteins, V and C, are also expressed from the genome in infected cells. The V protein is generated from a *P* gene transcript subpopulation possessing an insertion of a nontemplated G residue at a specific editing site, and hence the V protein consists of a P/V common amino-terminal half and a V unique carboxyl-terminal half. The V-unique C-terminal region contains 15 amino acid residues highly conserved among almost all paramyxoviruses, including 7 cysteine residues that form a zinc finger-like motif and, indeed, bind Zn^{2+} (Fukuhara et al., 2002; Huang et al., 2000; Liston and Briedis, 1994; Paterson et al., 1995; Steward et al., 1995). On the other hand, C′, C, Y1 and Y2 proteins, collectively called C proteins, are encoded by an overlapping, shifted open reading frame of the upstream regions of the P and V mRNAs with multiple translational start codons and a common termination codon. The C protein is expressed by viruses belonging to three of the five genera of *Paramyxovirinae*: *Respirovirus*, *Morbillivirus* and *Henipahvirus* (Lamb and Kolakofsky, 2001; Nagai, 1999; Nagai and Kato, 2004).

We demonstrated previously by using a reverse genetics system that SeV V and C proteins are categorically nonessential gene products for viral replication but that silencing their expression severely impairs viral replication and pathogenesis, and we have suggested that the V and C proteins function for evading host innate immunity (Kato et al., 1997a, 1997b; Kurotani et al., 1998). However, C-knockout (KO) SeV [rSeV C(−)] and V-KO SeV [rSeV V(−)] were different with respect to their replication in cultured cells and in the mouse lung: replication of rSeV C(−) was severely impaired both in cultured cells and in mice, whereas rSeV V(−) propagated in cultured cells as efficiently as or more efficiently than the parental wild-type (WT) virus but showed a unique attenuated replication phenotype in mice. V(−) virus replicated in the mouse lung as efficiently as WT virus until 1 day after infection but was cleared from the lung thereafter far more rapidly than WT virus, indicating that SeV V protein encodes a *luxury function* required for *in vivo* viral replication and pathogenesis (Kato et al., 1997a). The pathogenicity determinant in the V protein was mapped to the highly conserved cysteine-rich C-terminal half (Fukuhara et al., 2002; Huang et al., 2000; Kato et al., 1997b). The unique characteristics of rSeV V(−) replication have been confirmed with a highly virulent SeV field isolate (Sakaguchi et al., 2003). The poor replication capacity of rSeV C(−) both in cultured cells and in mice could be explained by the multifunctional capacity of the C protein, with probable roles in virus assembly (Hasan et al., 2000; Sugahara et al., 2004) and control of viral transcription (Curran et al., 1992), in addition to inhibition of IFN signaling. It is unclear, however, whether the *luxury function* of SeV V protein required for *in vivo* viral replication and pathogenesis can be explained by its inhibitory effect on IRF-3 activation leading to negative regulation of IFN-β production because rSeV V(−) can replicate well even in cultured cells producing IFN, and IRF-3 also functions as a key activator of multiple cellular genes other than the immediate early IFN-α/β genes (Grandvaux et al., 2002).

In the present study, we investigated replication and pathogenesis of rSeV mutants of the V protein in mice possessing various deficiencies in innate immunity to clarify roles of the inhibitory capacity of V protein against IRF-3 activation in virus replication and pathogenesis. The results obtained indicate that propagation of the rSeV mutants in the mouse lung is suppressed by a cellular factor(s) induced by IRF-3, but it is not IFN, and that SeV V protein is required for counteracting IRF-3-induced innate immunity other than IFN to replicate efficiently in mice.

Results

Inhibition of rSeV V(−) replication at the early phase of mouse infection occurs independently of T cell functions

To investigate the involvement of T cell functions in rSeV V(−) clearance from infected mice, replication of V(−) virus was examined in T cell-deficient BALB/$c^{nu/nu}$ mice. rSeV WT propagated efficiently in BALB/c mice and was cleared from the lung by day 9 post infection (p.i.) (Fig. 1A). In BALB/$c^{nu/nu}$ mice, WT virus replicated more efficiently than it did in BALB/c mice, and a high virus load was maintained throughout the observed infection period (Fig. 1B). In contrast, in BALB/c mice, V(−) virus began to be cleared from day 2 p.i. in a stepwise manner and became undetectable on day 7 p.i., though the infectivity of V(−) virus on the first day p.i. was almost the same as that of WT virus (Fig. 1A), confirming previous results obtained in ICR mice (Kato et al., 1997a). In BALB/$c^{nu/nu}$ mice, rSeV V(−) grew as well as WT virus until the first day p.i. and then decreased rapidly to approximately 1/1000 of WT virus in infectivity by day 3 p.i. (Fig. 1B), as was observed in BALB/c mice. However, infectivity of V(−) virus in the lung began to increase again from day 5 p.i., and the titers remained thereafter in the order of 10^6 cell-infecting units (CIU), less than 1/10 of that of WT virus. These results indicate that clearance of rSeV

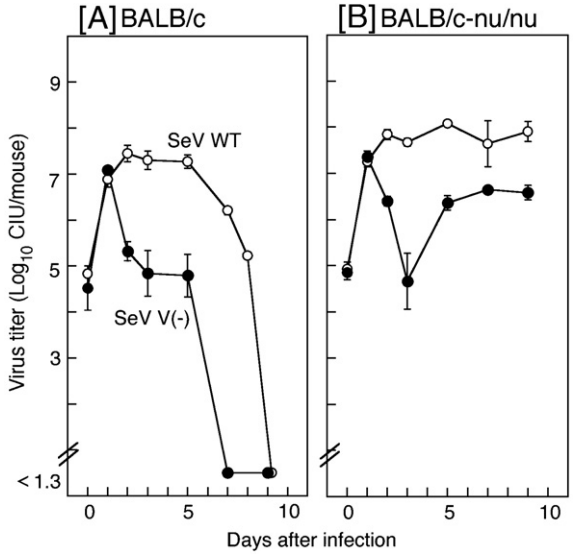

Fig. 1. Time course of rSeV V(−) replication in the lungs of T cell-deficient nude mice. Four-week-old BALB/c (A) and BALB/c$^{nu/nu}$ mice (B) were infected i.n. with 10^7 CIU of rSeV WT (open circles) or rSeV V(−) (closed circles). At the indicated intervals, three mice in each group were sacrificed and examined for virus replication in the lung. Each value represents the mean ± SE of 3 mice.

V(−) at the early phase of infection in BALB/c, BALB/c$^{nu/nu}$ and ICR mice occurs independently of T cell functions. It may be caused by an innate immunity, the activity of which appeared to peak on day 3 p.i. and persist thereafter at a low level (Fig. 1B), while the virus clearance in BALB/c and ICR mice at the later phase after day 5 p.i. (Fig. 1A) could be mainly due to specific adaptive immunity such as cytotoxic T lymphocytes.

Induction of IFN activity in rSeV V(−)-infected mice

Induction of IFN in the lungs of rSeV V(−)-infected ICR mice was examined by titration of antiviral activity against vesicular stomatitis virus (VSV) and by ELISA for IFN-γ to clarify the mechanism underlying the early clearance of V(−) virus from mice (Fig. 2). On the first day p.i., V(−) virus-infected mice showed activity levels of type I IFN several-fold higher than those in the WT virus-infected mice (Fig. 2B, see also Figs. 5A, C), although almost the same virus infectivities were detected in lungs of the V(−) and WT virus-infected mice (Fig. 2A), consistent with the fact that the V protein of SeV inhibits activation of IRF-3 and IFN-β induction in cells (Komatsu et al., 2004; Poole et al., 2002). IFN activities then decreased in the V(−) virus-infected mice in contrast to the increase in the WT virus-infected mice. This may be a reflection of the levels of virus propagation in the respective infected mice. On the other hand, levels of IFN-γ titers produced in the V(−) virus-infected mice were similar to or lower during the course of infection than those in the WT virus-infected mice (Fig. 2B). V(−) virus showed essentially the same replication pattern in BALB/c-IFN-γ$^{-/-}$ mice as that in BALB/c mice (data not shown). These results suggest that the observed early clearance of SeV V(−) from the mouse lung might be caused by the unusual strong induction of type I IFN and/or NK cell activities at the early phase of V(−) virus infection.

Replication of rSeV V(−) in NK cell-deficient beige mice

rSeV V(−) showed the unique early poor replication pattern even in C57BL/6$^{bg/bg}$ mice (Fig. 3B) as in C57BL/6 mice (Fig. 3A), although cytocidal activity of NK cells was deficient under the condition of SeV infection in the beige mice (Fig. 3C). The results indicate that the early clearance of V(−) virus from the mouse lung is independent of NK cell activity.

Replication and pathogenesis of rSeV V(−) and rSeV $V_{\Delta C}$ in IRF-3 KO mice

Since SeV V protein inhibits IRF-3 activation and IFN-β induction in infected cells, replication and pathogenesis of rSeV V(−) and rSeV $V_{\Delta C}$ (an SeV mutant expressing the V

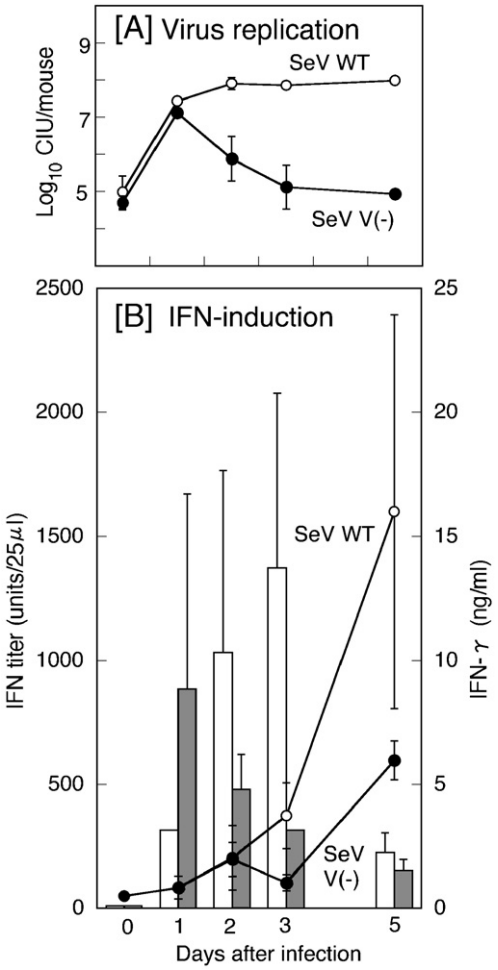

Fig. 2. Induction of IFN activity in rSeV V(−)-infected mice. Three-week-old ICR mice were infected i.n. with 10^7 CIU of rSeV WT or rSeV V(−), and virus replication (A) and IFN production (B) in the lung were examined. IFN activities in the lung homogenates of mice infected with rSeV WT (open bars) or rSeV V(−) (shaded bars) were measured biologically by titration of antiviral activity against VSV. IFN-γ produced in the lung infected with rSeV WT (open circles) or rSeV V(−) (closed circles) was assayed by ELISA. Each value represents the mean ± SE of 3 mice.

protein lacking the V-unique C terminal half) were investigated in IRF-3 KO mice to determine the involvement of IRF-3 in the early clearance of V mutant viruses from the lung. In the control C57BL/6J mice, rSeV V(−) and rSeV $V_{\Delta C}$ showed essentially the same early clearance phenotype as that observed in ICR, BALB/c and C57BL/6 mice (Fig. 4A), although rSeV $V_{\Delta C}$ was cleared from the lung more slowly than rSeV V(−), consistent with previous results obtained in ICR mice (Kato et al., 1997b). In C57BL/6J-IRF-$3^{-/-}$ mice, rSeV WT appeared to replicate more efficiently than it did in the control mice (Figs. 4A and B). Furthermore, in marked contrast to the control mice, both V(−) and $V_{\Delta C}$ viruses also propagated very efficiently in the KO mice and their unique early clearance phenotype was not observed (Fig. 4B). V(−) virus appeared, however, to show slightly less replication after 5 days p.i. than that of WT virus even in the KO mice, while $V_{\Delta C}$ virus replicated as well as WT virus (Fig. 4B).

Pathogenicity of the WT and V mutant viruses to mice, examined by mouse body weight loss and lung consolidation, paralleled well the respective virus replication ability in C57BL/6J and C57BL/6J-IRF-$3^{-/-}$ mice. Both V(−) virus-infected and $V_{\Delta C}$ virus-infected C57BL/6J mice showed less body weight loss and lung consolidation than those of WT virus-infected mice, although weight loss and lung consolidation of $V_{\Delta C}$ virus-infected mice were slightly greater than those of V(−) virus-infected mice (Fig. 4A). On the other hand, in the KO mice, V(−) virus and $V_{\Delta C}$ virus infection resulted in large degrees of body weight loss and lung consolidation similar to those caused by WT virus, and all of the infected mice died within the observation period (Fig. 4B). Table 1 shows comparisons of LD_{50}s of WT, V(−) and $V_{\Delta C}$ viruses in C57BL/6J mice and those in C57BL/6J-IRF-$3^{-/-}$ mice. The LD_{50}s of V(−) and $V_{\Delta C}$ viruses in C57BL/6J mice were 184-fold and 100-fold higher, respectively, than that of WT virus, while in the KO mice, LD_{50} of V(−) virus was 37-fold higher than that of WT virus and $V_{\Delta C}$ virus showed exactly the same LD_{50} as that of WT virus.

These results indicate that a certain gene product induced by IRF-3, including IFN-β, is involved in the early clearance of SeV V(−) and SeV $V_{\Delta C}$ from the lung and viral pathogenesis and also that $V_{\Delta C}$ virus, but not V(−) virus, possesses the capacity to replicate as efficiently as WT virus in IRF-3 KO mice.

Production of IFN in IRF-3 KO mice infected with SeV

To confirm the absence of induction of IFN through IRF-3 activation, type I IFN production was examined in C57BL/6J-IRF-$3^{-/-}$ mice infected with rSeV WT or rSeV V(−) (Fig. 5). In the control C57BL/6J mice infected with WT or V(−) virus, essentially the same results as those shown in Fig. 2 were obtained (Fig. 5A). However, high titers of IFN activity were also detected in the WT virus- or V(−) virus-infected IRF-3 KO mice (Fig. 5D), although kinetics of IFN induction was significantly different from that in C57BL/6J mice. The IFN activity in WT virus-infected KO mice peaked on day 2 p.i. and disappeared by day 7 p.i., while the peak of IFN activity in infected C57BL/6J mice was on day 3 p.i. and high titers of the activity were detected until day 7 p.i. In addition, unusual strong induction of IFN activity in the V(−) virus-infected C57BL/6J mice on day 1 p.i. compared with that in the WT virus-infected mice was not observed in the KO mice. When IFN-α/β were measured by ELISA, both peaks of IFN-α and IFN-β were on day 2 p.i. in the WT or V(−) virus-infected IRF-3 KO mice (Figs. 5E, F), while in the WT virus-infected C57BL/6J mice, the peaks of IFN-β and IFN-α were on day 1 p.i. and day 3 p.i., respectively (Fig. 5B). In the V(−) virus-infected C57BL/6J mice, both peaks of IFN-β and IFN-α were on day 1 p.i., and the titer of IFN-β was several-fold higher than that in the WT virus-infected mice (Fig. 5C). Subsequent increase in IFN-α was not detected in the V(−) virus-infected C57BL/6J mice. These results suggest that the IFN-α/β detected in the KO mice are produced through a pathway other than IRF-3-mediated signal transduction. It is possible that IFN was induced through

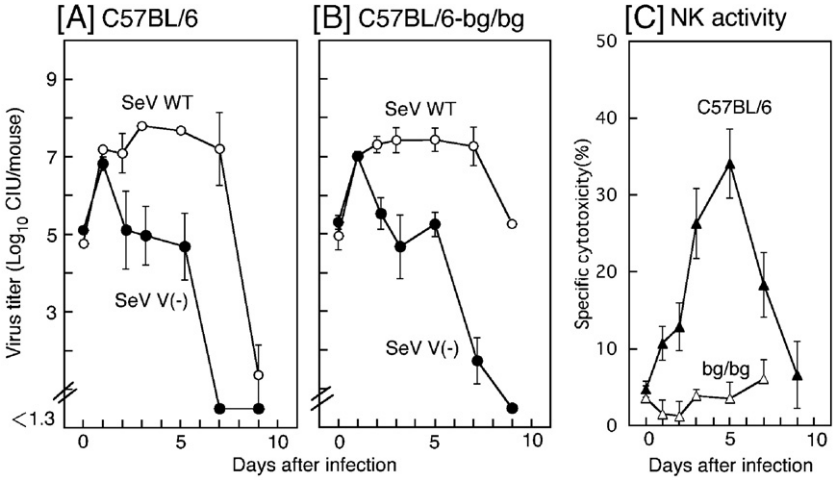

Fig. 3. Replication of rSeV V(−) in NK cell-deficient beige mice. Five-week-old C57BL/6 (A) and C57BL/$6^{bg/bg}$ (beige) (B) mice were infected i.n. with 10^7 CIU of rSeV WT (open circles) or rSeV V(−) (closed circles), and viral infectivities in the lung were assayed at various time points after infection. Splenic NK cell activity (C) was measured in the C57BL/6 (closed triangles) and beige mice (open triangles) infected with 10^7 CIU of rSeV WT using the standard ^{51}Cr-release assay method against YAC-1 target cells, and specific cytotoxicity (%) was determined at an R/T ratio of 50:1. Each value represents the mean ± SE of 3 mice.

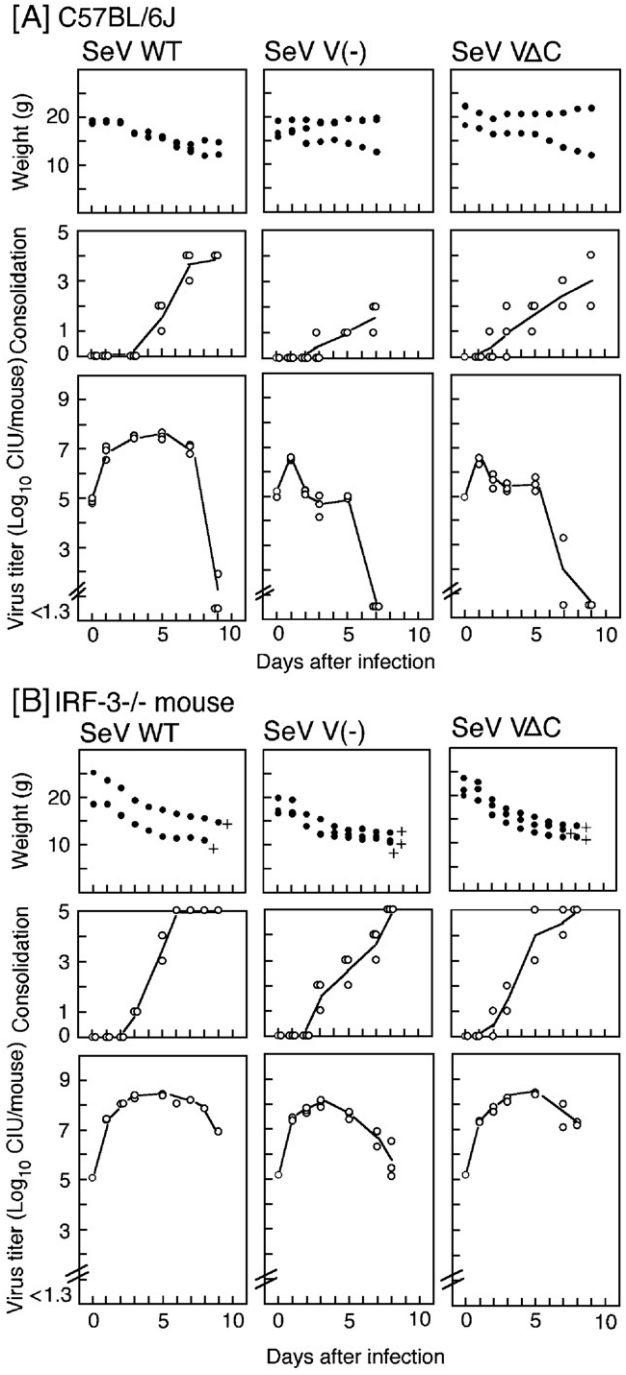

Fig. 4. Replication and pathogenicity of rSeV V(−) and rSeV $V_{\Delta C}$ in IRF-3 KO mice. Five-week-old C57BL/6J (A) and C57BL/6J-IRF-$3^{-/-}$ mice (B) were infected i.n. with 10^7 CIU of rSeV WT, rSeV V(−) or SeV $V_{\Delta C}$. The mice were weighed daily, and 2 or 3 mice in each group were sacrificed at the indicated intervals and examined for lung consolidation and virus infectivity in the lung. Each circle shows one mouse and + indicates a mouse that died.

activation of IRF-7, which was constitutively or basally expressed in plasmacytoid dendritic cells (pDCs) in the mice (Honda et al., 2005; Izaguirre et al., 2003). We therefore examined IFN induction in cultures of primary mouse fibroblast cells derived from C57BL/6J and C57BL/6J-IRF-$3^{-/-}$ mice, in which pDCs were absent. As shown in Table 2, neither IFN-β nor IFN-α was detected in the cultures of cells derived from the IRF-3 KO mouse upon infection with WT or V(−) virus, in contrast to production of significant amounts of IFN-α/β in the cell cultures from C57BL/6J mice. These results indicate that high titers of type I IFN are produced even in the IRF-3 KO mouse by SeV infection, probably through IRF-7 activation in pDCs, thus suggesting that IFN might not account for the early clearance of SeV V mutants from the mouse lung.

Replication of rSeV V(−) in mice deficient in IFN signal transduction

To further investigate whether the strong induction of type I IFN observed in rSeV V(−)-infected mice is directly involved in the virus clearance at the early phase of infection, virus replication in the lung was examined in IFN signal transduction-deficient mice, IFN-α/β receptor KO mice and STAT1 KO mice. V (−) virus showed the unique early clearance phenotype even in IFN-α/β $R^{-/-}$ (A129) mice, similar to that in 129/Sv mice (Fig. 6). Essentially the same results were also obtained in $STAT1^{-/-}$ mice (data not shown). These results, together with the results described in the preceding section, indicate that IFN-α/β is not a main effector for the early clearance of V(−) virus from the mouse lung and suggest that a certain factor, other than IFN-β, induced through IRF-3 activation is involved in the early virus clearance.

Discussion

In the present study, we investigated the replication and pathogenesis of SeV V mutants, rSeV V(−) and rSeV $V_{\Delta C}$, using mice with various deficiencies in innate immunity to clarify the *in vivo* significance of the inhibitory capacity of V protein against IRF-3 activation, particularly whether IFN is directly involved in the clearance of the SeV mutants from the lung at the early stage of infection. Consistent with the prediction by results obtained *in vitro* showing suppression of IFN-β induction through inhibition of IRF-3 activation by the V protein (He et al., 2002; Komatsu et al., 2004; Poole et al., 2002), a several-fold higher level of IFN was actually produced on day 1 p.i. in the rSeV V(−)-infected mouse lung compared with that in the WT virus-infected mice (Figs. 2 and 5A, C). In addition, early clearance of the SeV mutants did not occur in the IRF-3 KO mice (Fig. 4), suggesting that IFN induced through IRF-3 activation may account for the early clearance of the virus mutants. However, high titers of IFN-α/β, probably produced from pDCs through the TLR9/7-Myd88-IRF-7 pathway (Honda et al., 2005), were detected on day 2 p.i. even in the IRF-3 KO mice

Table 1
LD_{50}s of rSeV mutants in IRF-3 KO mice

Virus	LD_{50} (CIU/mouse) in	
	C57BL/6J	C57BL/6J-IRF-$3^{-/-}$
rSeV WT	3.2×10^5 (1)	1.5×10^4 (1)
rSeV V(−)	5.9×10^7 (184)	5.6×10^5 (37)
rSeV $V_{\Delta C}$	3.2×10^7 (100)	1.5×10^4 (1)

The numbers in parentheses are ratios of LD_{50} of rSeV V(−) and rSeV $V_{\Delta C}$ to that of rSeV WT in C57BL/6J or C57BL/6J-IRF-$3^{-/-}$ mice.

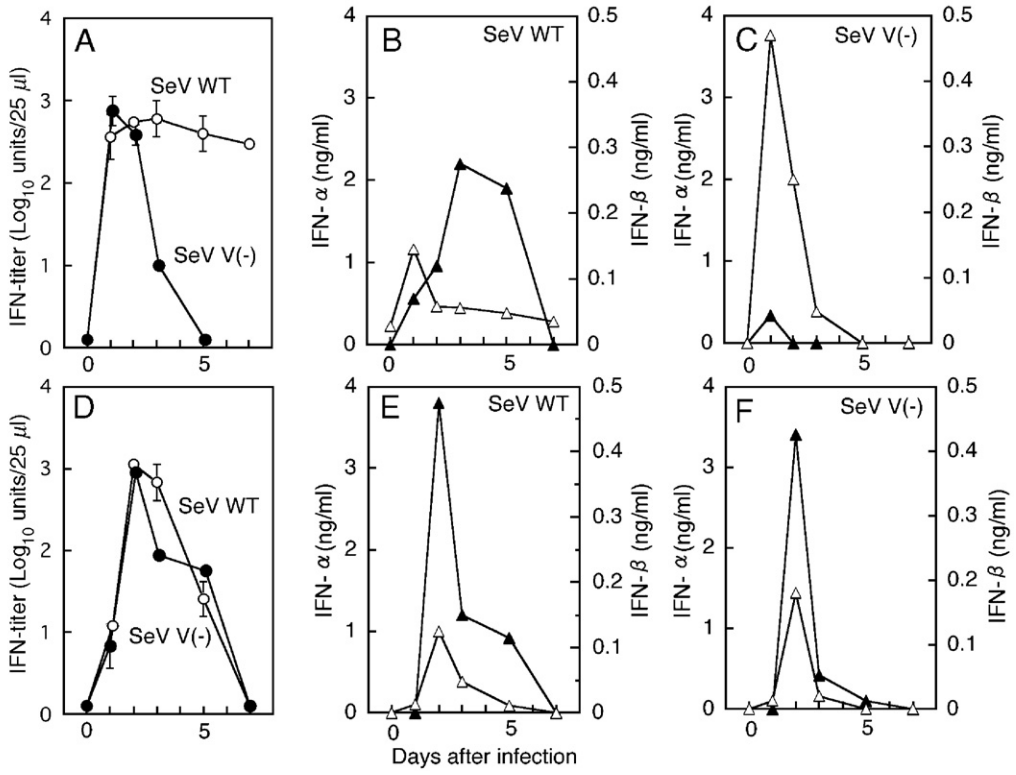

Fig. 5. IFN production in IRF-3 KO mice infected with SeV. Five-week-old C57BL/6J (A, B, C) or IRF-3$^{-/-}$ mice (D, E, F) were infected i.n. with 10^7 CIU of rSeV WT or rSeV V(−), and IFN induction in the lung at various time points after infection was determined. (A, D) IFN activities in the lung were titrated biologically after infection with rSeV WT (open circles) or rSeV V(−) (closed circles). Each value represents the mean±SE of 2 or 3 mice. (B, E) IFN-α (closed triangles) and IFN-β (open triangles) in the WT virus-infected mouse lung were assayed by ELISA. (C, F) IFN-α (closed triangles) and IFN-β (open triangles) in the V(−) virus-infected mouse lung were also assayed by ELISA.

infected with rSeV or rSeV V(−) (Figs. 5D, E, F), wherein the SeV mutants were able to replicate almost normally (Fig. 4B). Furthermore, early clearance of the SeV mutants was also observed in mice deficient of IFN signal transduction, IFN-α/β receptor KO mice (Fig. 6) and STAT1 KO mice. These results indicate that the host factor responsible for the early clearance of the SeV mutants is expressed through activation of IRF-3 but that it is not IFN. Therefore, the major role of SeV V protein in *in vivo* viral replication and pathogenesis is thought to be counteraction of a certain antiviral innate immunity other than IFN induced by IRF-3 rather than inhibition of IFN production, but the possibility of involvement of IFN and NK cells in the early clearance of the virus mutants as indirect or supplementary effectors could not be ruled out. Although the IFN response seems not to be important in controlling early SeV replication *in vivo*, it should be noted that the SeV V mutants used still have functional C proteins to help counter the IFN response.

Table 2
IFN production in rSeV WT or rSeV V(−)-infected primary fibroblast cultures prepared from C57BL/6J and C57BL/6J-IRF-3$^{-/-}$ mice

Cells	Infected with	IFN production (pg/ml)	
		IFN-α	IFN-β
C57BL/6J	WT	450	560
	V(−)	420	1400
IRF-3$^{-/-}$	WT	<1	<1
	V(−)	<1	<1

Monolayers of primary mouse fibroblasts prepared from C57BL/6J or C57BL/6J-IRF-3$^{-/-}$ mice were infected with rSeV WT or rSeV V(−) at an m.o.i. of approximately 10, and IFN-α and IFN-β in the medium at 24 h p.i. were assayed by ELISA.

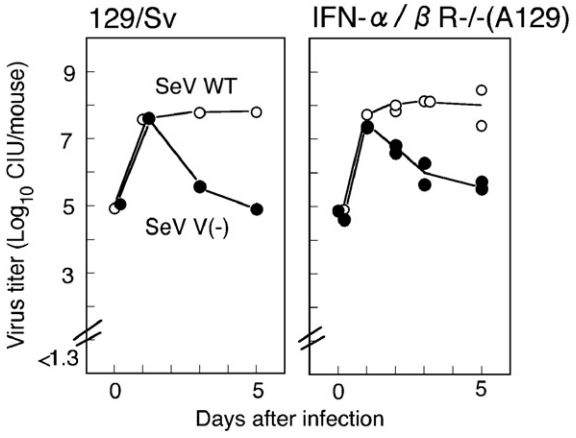

Fig. 6. Replication of rSeV V(−) in the lung of IFN-α/β receptor KO mice. Six- to seven-week-old IFN-α/β R$^{-/-}$ (A129) mice and their parent 129/Sv mice were infected i.n. with 10^7 CIU of rSeV WT (open circles) or rSeV V(−) (closed circles), and viral infectivities in the lung were measured at the indicated time points after infection. Each point shows one mouse.

As for the reason why rSeV V(−) showed slightly less replication ability and pathogenicity than those of rSeV $V_{\Delta C}$ in the IRF-3 KO mice (Fig. 4B and Table 1), it is likely that the IFN-γ produced in the infected mice (Fig. 2) accounts for the difference between the mutant viruses. rSeV $V_{\Delta C}$ might be able to propagate in the KO mice as efficiently as WT virus by anti-IFN-γ activity of the N-terminal P/V common fragments of V protein, which were expressed in the $V_{\Delta C}$ virus-, but not the V(−) virus-, infected cells since the N-terminal P/V common fragments, but not the C-terminal V unique fragments, constitutively expressed in HeLa cells showed significant inhibitory activities against antiviral effects of IFN-γ (A. Kato, unpublished data), although the V protein and its P/V common and V unique fragments did not show any anti-IFN-α/β activity (Kato et al., 2004). This view is also supported by the observation of lower replication of V(−) virus after day 5 p.i. in the KO mice (Fig. 4B), compared to that of $V_{\Delta C}$ virus, when significant IFN-γ production was induced.

The present study suggested that expression of the host factor responsible for the early clearance of the SeV V mutants from mice is closely related to the functions of IRF-3 other than the induction of IFN-β. Many IFN-stimulated responsive element (ISRE)-containing genes are induced in response to virus infection without the need for prior *de novo* IFN synthesis (Grandvaux et al., 2002; Nakaya et al., 2001; Nicholl et al., 2000). IRF-3 is ubiquitously present in a latent form in the cytoplasm of uninfected cells and upon stimulation mediates gene transcription through recognition of ISRE sequences. Thus, IRF-3 is considered a potential candidate to regulate IFN-stimulated genes (ISGs) in the early events of innate response to virus infection. It has been reported that activated IRF-3 transcriptionally upregulates the chemokine genes *RANTES* and *IP-10* (Lin et al., 1999; Nakaya et al., 2001), and other ISGs, such as *ISG15*, *ISG54*, *ISG56*, *ISG60*, *Arg II*, *GBP1* and *CIG5* (Grandvaux et al., 2002; Nakaya et al., 2001), in addition to *IFN-β*. Among these, *ISG15* (Lenschow et al., 2005; Ritchie et al., 2004), *GBP1* (Anderson et al., 1999) and *CIG5* (Chin and Cresswell, 2001) gene products have been demonstrated to possess antiviral activity. Furthermore, IRF-3 plays a role in mediating SeV-induced apoptosis (Heylbroeck et al., 2000). It has recently been shown that the *Arg II* gene product (arginase II) participates in the IFN-independent antiviral response through polyamine production and induction of apoptosis (Grandvaux et al., 2005). Involvement of arginase II in the early clearance of the SeV V mutants through apoptosis induction, however, seems unlikely because V(−) virus was able to propagate in cultured cells as efficiently as or more efficiently than WT virus, although enhanced cytopathic effects (CPE), possibly due to apoptosis, were actually observed in the cells (Kato et al., 1997a). The host factor responsible for the early clearance of the SeV mutants expressed through IRF-3 activation remains to be elucidated.

The mechanism underlying the *luxury function* of SeV V protein, which is required for viral replication and pathogenesis *in vivo* but not in cultured cells, is still enigmatic. The host factor responsible for the early clearance of the SeV V mutants might be expressed in cells present only in mice, such as macrophages and DCs, through IRF-3 activation but not in usual cell lines. Alternatively, since V(−) virus propagated as well as WT virus until 1 day after infection in the mouse lung (Fig. 1), a host factor other than IFN-β expressed through IRF-3 activation by virus infection might *trans*-activate some cells present only in mice to exert antiviral activity or to produce another host factor for the innate virus clearance.

Studies on SeV accessory proteins V and C, together with the present study, have shown that the V and C proteins play important roles in evading host innate immunity cooperatively. SeV V and C proteins might have differentiated to counteract the unknown IRF-3-mediated innate immunity and the IFN system, respectively, from the common ancestral V protein of paramyxoviruses possessing both anti-IRF-3 and anti-IFN signaling activities such as SV5 V protein. The function of SeV V protein revealed in the present study has been shown only with SeV at present, but it is possible that other paramyxovirus V proteins also possess such a function since the V-unique C-terminal half responsible for counteracting the early clearance of SeV from mice is highly conserved among paramyxoviruses, and all of the paramyxovirus V proteins of SV5, human parainfluenza virus 2, mumps virus, Hendra virus as well as SeV have been shown to inhibit IRF-3 activation through binding to mda-5 (Andrejeva et al., 2004). Studies on the V protein of SeV, for which an experimental animal model is available, will shed light on innate immunity against virus infection.

Materials and methods

Cells and viruses

LLC-MK_2, Vero, BHK-21 and L cells were grown in Eagle's MEM supplemented with 10% bovine serum (BS). NK cell-sensitive YAC-1 cells, derived from Moloney murine leukemia virus-induced lymphoma in an A/Sn mouse, were grown in Iscove-modified DMEM supplemented with 10% FCS. Primary mouse fibroblast cells were prepared from 1- or 2-day-old newborn C57BL/6J or C57BL/6J IRF-$3^{-/-}$ mice by trypsinization and grown in DMEM supplemented with 10% FCS.

rSeV WT and its mutant viruses rSeV V(−) and rSeV $V_{\Delta C}$ recovered from cDNAs (Kato et al., 1996, 1997a, 1997b) were used in the present study, and they were propagated in 11-day-old embryonated chicken eggs. Infectivity was measured by an immunofluorescent cell-counting assay using LLC-MK_2 cells as host cells and expressed as CIU per ml (Kiyotani et al., 1990). VSV-NJ strain was grown in BHK-21 cells, and the infectivity was measured by the tissue culture 50% infectious dose ($TCID_{50}$) method using L cells.

Infection of animals

Three-week-old male ICR mice, 4-week-old male BALB/c and BALB/$c^{nu/nu}$ (nude) mice and 5-week-old male C57BL/6 and C57BL/$6^{bg/bg}$ (beige) mice were purchased from Charles River Japan, Inc. (Atsugi, Japan). Six- to seven-week-old male

IFN-α/β $R^{-/-}$ mice (A129) and their parent mice (129/Sv) were purchased from B&K Ltd. (East Yorkshire, UK). Four- to six-week-old BALB/c-IFN-γ$^{-/-}$ mice were kind gifts from Dr. Yoichiro Iwakura (The Institute of Medical Sciences, The University of Tokyo, Japan). Five-week-old male STAT1$^{-/-}$ mice (Taconic 002045-M) and their parent mice 129S6 (Taconic 129SVE) were purchased from Taconic Quality Laboratory Animals and Service for Research (Petersburg, NY). Mating pairs of C57BL/6J-IRF-3$^{-/-}$ mice (No. 00858) were purchased from RIKEN Bioresource Center (Ibaraki, Japan), and the offspring of both sexes were used for experiments at the age of 5 weeks. Five-week-old C57BL/6J mice were purchased from CREA Japan Inc. (Tokyo, Japan) and used as background controls of IRF-3 KO mice. Deficiency of IFN-α/β receptor in A129 mice was indirectly checked by unresponsiveness of primary lung cells, derived from A129 mice, to IFN treatment for protection of the cells from VSV challenge (data not shown). Lack of STAT1 expression in the STAT1$^{-/-}$ mice was confirmed by Western blotting using an anti-STAT1 antibody (data not shown). Deficiency of IRF-3 expression in the IRF-3$^{-/-}$ mice was also confirmed by Western blotting using an anti-IRF-3 antibody (data not shown).

All of the mice used were specific pathogen-free and were kept under bioclean and regulated conditions in the BSL3 facility in the Institute of Laboratory Animal Science, Division for Research Support, Life Science Center, Hiroshima University. All animal experiments complied with the guidelines set by the Institute of Laboratory Animal Science, Hiroshima University. Each mouse was infected intranasally (i.n.) with a 25-μl inoculum containing viruses of 10^7 CIU under anesthetization with inhalation of ether and/or i.p. injection of Nembutal. Infected mice were checked daily for body weight and clinical signs, and at intervals, some of them were sacrificed for investigation of lung consolidation, virus replication and IFN production in the lung, and NK cell activity of splenic lymphocytes. Lung consolidation was graded from 1 to 4 according to the extent of macroscopic lung lesion, and one point was added when the mouse died (Kato et al., 1997a). Virus replication in the mouse lung was determined according to the method described previously (Kiyotani et al., 1990). LD_{50}s of rSeV WT, rSeV V(−) or rSeV $V_{\Delta C}$ were determined as described previously (Kiyotani et al., 1990). Five mice in a group of 5-week-old C57BL/6J or C57BL/6J-IRF-3$^{-/-}$ mice were infected i.n. with various doses of respective SeV and observed for 2 weeks, and LD_{50}s were then calculated by the method of Reed and Muench.

IFN assay

IFN activities of SeV-infected mouse lung homogenates were determined biologically according to the method described previously (Kiyotani et al., 1990). IFN-α, IFN-β and IFN-γ were measured by ELISA using a Mouse Interferon Alpha ELISA kit, Mouse Interferon Beta ELISA kit (PBL Biomedical Laboratories, Piscataway, NJ) or Mouse IFN-γ Immunoassay kit (R&D Systems, Minneapolis, MN) according to the manufacturer's instructions.

Assay of NK cell activity

NK cell activities of SeV-infected mouse splenic lymphocytes were measured using the standard ^{51}Cr-release assay method against YAC-1 target cells as described previously (Kiyotani et al., 1990), and the specific cytotoxicity (%) at an E/T ratio of 50:1 was determined.

Western blotting analysis of IRF-3 and STAT1

Western blotting was performed as described previously (Sakaguchi et al., 1999) with some modifications. Liver emulsions were prepared from 5-week-old C57BL/6J-IRF-3$^{-/-}$ or C57BL/6J mice, resolved by SDS-PAGE using a 10% gel, and transferred onto a polyvinylidene difluoride membrane (Amersham Biosciences, Piscataway, NJ). The membrane was probed with an anti-IRF-3 antibody (sc-9082; Santa Cruz Biotechnology, Santa Cruz, CA) or an anti-actin antibody (MAB1501; Chemicon, Temecula, CA) for an internal control and subsequently with peroxidase-conjugated anti-mouse IgG antibody followed by visualization with an ECL Advance Western Blotting Detection System (Amersham Biosciences). For detection of STAT1, spleen emulsions were prepared from 5-week-old STAT1$^{-/-}$ or 129S6 mice, and the protein-blotted membrane was probed with an anti-STAT1 antibody (sc-346; Santa Cruz Biotechnology).

Acknowledgments

We thank Dr. Y. Iwakura (The Institute of Medical Sciences, The University of Tokyo) for providing BALB/c-IFN-γ$^{-/-}$ mice. This work was supported by grants-in-aid for scientific research from the Japan Society for the Promotion of Science and the Ministry of Education, Culture, Sports, Science, and Technology.

References

Anderson, S.L., Carton, J.M., Lou, J., Xing, L., Rubin, B.Y., 1999. Interferon-induced guanylate binding protein-1 (GBP-1) mediates an antiviral effect against vesicular stomatitis virus and encephalomyocarditis virus. Virology 256, 8–14.

Andrejeva, J., Childs, K.S., Young, D.F., Carlos, T.S., Stock, N., Goodbourn, S., Randall, R.E., 2004. The V proteins of paramyxoviruses bind the IFN-inducible RNA helicases, mda-5, and inhibit its activation of the IFN-β promoter. Proc. Natl. Acad. Sci. U.S.A. 101, 17264–17269.

Chin, K.-C., Cresswell, P., 2001. Viperin (cig5), an IFN-inducible antiviral protein directly induced by human cytomegalovirus. Proc. Natl. Acad. Sci. U.S.A. 98, 15125–15130.

Curran, J., Marq, J.B., Kolakofsky, D., 1992. The Sendai virus nonstructural C proteins specifically inhibit viral mRNA synthesis. Virology 189, 647–656.

Didcock, L., Young, D.F., Goodbourn, S., Randall, R.E., 1999a. Sendai virus and simian virus 5 block activation of interferon-responsive gene: importance for virus pathogenesis. J. Virol. 73, 3125–3133.

Didcock, L., Young, D.F., Goodbourn, S., Randall, R.E., 1999b. The V protein of simian virus 5 inhibits interferon signaling by targeting STAT1 for proteasome-mediated degradation. J. Virol. 73, 9928–9933.

Fujii, Y., Sakaguchi, T., Kiyotani, K., Huang, C., Fukuhara, N., Egi, Y., Yoshida, T., 2002. Involvement of the leader sequence in Sendai virus pathogenesis revealed by recovery of a pathogenic field isolate from cDNA. J. Virol. 76, 8540–8547.

Fukuhara, N., Huang, C., Kiyotani, K., Yoshida, T., Sakaguchi, T., 2002.

Mutational analysis of the Sendai virus V protein: importance of the conserved residues for Zn binding, virus pathogenesis, and efficient RNA editing. Virology 299, 181–182.
Garcia-Sastre, A., 2001. Inhibition of interferon-mediated antiviral responses by influenza A viruses and other negative-strand RNA viruses. Virology 279, 375–384.
Garcia-Sastre, A., 2004. Identification and characterization of viral antagonists of type I interferon in negative-strand RNA viruses. Curr. Top. Microbiol. Immunol. 283, 249–280.
Garcin, D., Latorre, P., Kolakofsky, D., 1999. Sendai virus C proteins counteract the interferon-mediated induction of an antiviral state. J. Virol. 73, 6559–6565.
Goodbourn, S., Didcock, L., Randall, R.E., 2000. Interferons: cell signaling, immune modulation, antiviral responses and virus countermeasures. J. Gen. Virol. 81, 2341–2364.
Gotoh, B., Takeuchi, K., Komatsu, T., Yokoo, J., Kimura, Y., Kurotani, A., Kato, A., Nagai, Y., 1999. Knockout of the Sendai virus C gene eliminates the viral ability to prevent the interferon-alpha/beta-mediated responses. FEBS Lett. 459, 205–210.
Gotoh, B., Komatsu, T., Takeuchi, K., Yokoo, J., 2001. Paramyxovirus accessory proteins as interferon antagonists. Microbiol. Immunol. 45, 787–800.
Gotoh, B., Komatsu, T., Takeuchi, K., Yokoo, J., 2002. Paramyxovirus strategies for evading the interferon response. Rev. Med. Virol. 12, 337–357.
Gotoh, B., Komatsu, T., Takeuchi, K., Yokoo, J., 2003a. The C-terminal half-fragment of the Sendai virus C protein prevents the gamma-activated factor from binding to a gamma-activated sequence site. Virology 316, 29–40.
Gotoh, B., Komatsu, T., Takeuchi, K., Yokoo, J., 2003b. The STAT2 activation process is a crucial target of Sendai virus C protein for the blockade of alpha interferon signaling. J. Virol. 77, 3360–3370.
Grandvaux, N., Servant, M.J., tenOever, B., Sen, G.C., Balachandran, S., Barber, G.N., Lin, R., Hiscott, J., 2002. Transcriptional profiling of interferon regulatory factor 3 target genes: direct involvement in the regulation of interferon-stimulated genes. J. Virol. 76, 5532–5539.
Grandvaux, N., Gaboriau, F., Harris, J., tenOever, B.R., Lin, R., Hiscott, J., 2005. Regulation of arginase II by interferon regulatory factor 3 and the involvement of polyamines in the antiviral response. FEBS J. 272, 3120–3131.
Hasan, M.K., Kato, A., Muranaka, M., Yamaguchi, R., Sakai, Y., Hatano, I., Tashiro, M., Nagai, Y., 2000. Versatility of the accessory C proteins of Sendai virus: contribution to virus assembly as an additional role. J. Virol. 74, 5619–5628.
He, B., Paterson, R.G., Stock, N., Durbin, J.E., Durbin, R.K., Goodbourn, S., Randall, R.E., Lamb, R.A., 2002. Recovery of paramyxovirus simian virus 5 with a V protein lacking the conserved cysteine-rich domain: the multifunctional V protein blocks both interferon-beta induction and interferon signaling. Virology 303, 15–32.
Heylbroeck, C., Balachandran, S., Servant, M.J., DeLuca, C., Barber, G.N., Lin, R., Hiscott, J., 2000. The IRF-3 transcription factor mediates Sendai virus-induced apoptosis. J. Virol. 74, 3781–3792.
Horvath, C.M., 2004. Weapons of STAT destruction. Interferon evasion by paramyxovirus V protein. Eur. J. Biochem. 271, 4621–4628.
Honda, K., Yanai, H., Negishi, H., Asagiri, M., Sato, M., Mizutani, T., Shimada, N., Ohba, Y., Takaoka, A., Yoshida, N., Taniguchi, T., 2005. IRF-7 is the master regulator of type I interferon-dependent immune responses. Nature 434, 772–777.
Huang, C., Kiyotani, K., Fujii, Y., Fukuhara, N., Kato, A., Nagai, Y., Yoshida, T., Sakaguchi, T., 2000. Involvement of the zinc-binding capacity of Sendai virus V protein in viral pathogenesis. J. Virol. 74, 7834–7841.
Ishida, N., Homma, M., 1978. Sendai virus. Adv. Virus Res. 23, 349–383.
Izaguirre, A., Barnes, B.J., Amrute, S., Yeow, W.-S., Megjugorac, N., Dai, J., Feng, D., Chung, E., Pitha, P.M., Fitzgerald-Bocarsly, P., 2003. Comparative analysis of IRF and IFN-alpha expression in human plasmacytoid and monocyte-derived dendritic cells. J. Leukoc. Biol. 74, 1125–1138.
Kato, A., Sakai, Y., Shioda, T., Kondo, T., Nakanishi, M., Nagai, Y., 1996. Initiation of Sendai virus replication from transfected cDNA or RNA with negative or positive sense. Genes Cells 1, 569–579.
Kato, A., Kiyotani, K., Sakai, Y., Yoshida, T., Nagai, Y., 1997a. The paramyxovirus, Sendai virus, V protein encodes a luxury function required for viral pathogenesis. EMBO J. 16, 578–587.
Kato, A., Kiyotani, K., Sakai, Y., Yoshida, T., Nagai, Y., 1997b. Importance of the cysteine-rich carboxyl-terminal half of V protein for Sendai virus pathogenesis. J. Virol. 71, 7266–7272.
Kato, A., Cortese-Grogan, C., Moyer, S.A., Sugahara, F., Sakaguchi, T., Kubota, T., Otsuki, N., Kohase, M., Tashiro, M., Nagai, Y., 2004. Characterization of the amino acid residues of Sendai virus C protein that are critically involved in its interferon antagonism and RNA synthesis down-regulation. J. Virol. 78, 7443–7454.
Kiyotani, K., Takao, S., Sakaguchi, T., Yoshida, T., 1990. Immediate protection of mice from lethal wild-type Sendai virus (HVJ) infections by a temperature-sensitive mutant, HVJpi, possessing homologous interfering capacity. Virology 177, 65–74.
Komatsu, T., Takeuchi, K., Yokoo, J., Tanaka, Y., Gotoh, B., 2000. Sendai virus blocks alpha interferon signaling to signal transducers and activator of transcription. J. Virol. 74, 2477–2480.
Komatsu, T., Takeuchi, K., Yokoo, J., Gotoh, B., 2002. Sendai virus C protein impairs both phosphorylation and dephosphorylation processes of Stat1. FEBS Lett. 511, 139–144.
Komatsu, T., Takeuchi, K., Yokoo, J., Gotoh, B., 2004. C and V proteins of Sendai virus target signaling pathways leading to IRF-3 activation for the negative regulation of interferon-β production. Virology 325, 137–148.
Kurotani, A., Kiyotani, K., Kato, A., Shioda, T., Sakai, Y., Mizumoto, K., Yoshida, T., Nagai, Y., 1998. Sendai virus C proteins are categorically nonessential gene products but silencing their expression severely impairs viral replication and pathogenesis. Genes Cells 3, 111–124.
Lamb, R.A., Kolakofsky, D., 2001. Paramyxoviridae: the viruses and their replication, 4th ed. In: Knipe, D.M., Howley, P.M., et al. (Eds.), Fields Virology, vol. 1. Lippincott Williams and Wilkins, Philadelphia, pp. 1305–1340.
Lenschow, D.J., Giannakopoulos, N.V., Gunn, L.J., Johnston, C., O'Guin, A.K., Schmidt, R.E., Levine, B., Virgin IV, H.W., 2005. Identification of interferon-stimulated gene 15 as an antiviral molecule during Sindbis virus infection in vivo. J. Virol. 79, 13974–13983.
Lin, R., Heylbroeck, C., Genin, P., Pitha, P.M., Hiscott, J., 1999. Essential role of interferon regulatory factor 3 in direct activation of RANTES chemokine transcription. Mol. Cell. Biol. 19, 959–966.
Liston, P.D., Briedis, J., 1994. Measles virus V protein binds zinc. Virology 198, 399–404.
Nagai, Y., 1999. Paramyxovirus replication and pathogenesis. Reverse genetics transforms understanding. Rev. Med. Virol. 9, 83–99.
Nagai, Y., Kato, A., 2004. Accessory genes of the Paramyxoviridae, a large family of nonsegmented negative strand RNA viruses, as a focus of active investigation by reverse genetics. Curr. Top. Microbiol. Immunol. 283, 198–248.
Nakaya, T., Sato, M., Hata, N., Asagiri, M., Suemori, H., Noguchi, S., Tanaka, N., Taniguchi, T., 2001. Gene induction pathways mediated by distinct IRFs during viral infection. Biochem. Biophys. Res. Commun. 283, 1150–1156.
Nicholl, M.J., Robinson, L.H., Preston, C.M., 2000. Activation of cellular interferon-responsive genes after infection of human cells with herpes simplex virus type 1. J. Gen. Virol. 81, 2215–2218.
Paterson, R.G., Leser, G.P., Shaughnessy, M.A., Lamb, R.A., 1995. The paramyxovirus SV5 V protein binds two atoms of zinc and is a structural component of virions. Virology 208, 121–131.
Poole, E., He, B., Lamb, R.A., Randall, R.E., Goodbourn, S., 2002. The V proteins of simian virus 5 and other paramyxoviruses inhibit induction of interferon-beta. Virology 303, 33–46.
Ritchie, K.J., Hahn, C.S., Kim, K., Yan, M., Rosario, D., Li, L., de la Torre, J.C., Zhang, D.-E., 2004. Role of ISG15 protease UBP43 (USP18) in innate immunity to viral infection. Nat. Med. 10, 1374–1378.
Sakaguchi, T., Uchiyama, T., Fujii, Y., Kiyotani, K., Kato, A., Nagai, Y., Kawai, A., Yoshida, T., 1999. Double-layered membrane vesicles released from mammalian cells infected with Sendai virus expressing the matrix protein of vesicular stomatitis virus. Virology 263, 230–243.
Sakaguchi, T., Kiyotani, K., Watanabe, H., Huang, C., Fukuhara, N., Fujii, Y., Shimazu, Y., Sugahara, F., Nagai, Y., Yoshida, T., 2003. Masking of the contribution of V protein to Sendai virus pathogenesis in a infection model with a highly virulent field isolate. Virology 313, 581–587.

Sen, G.C., 2001. Viruses and interferons. Annu Rev. Microbiol. 55, 255–281.
Steward, M., Samson, A.C., Errington, W., Emmerson, P.T., 1995. The Newcastle disease virus V protein binds zinc. Arch. Virol. 140, 1321–1328.
Sugahara, F., Uchiyama, T., Watanabe, H., Shimazu, Y., Kuwayama, M., Fujii, Y., Kiyotani, K., Adachi, A., Kohno, N., Yoshida, T., Sakaguchi, T., 2004. Paramyxovirus Sendai virus-like particle formation by expression of multiple viral proteins and acceleration of its release by C protein. Virology 325, 1–10.
Takeuchi, K., Komatsu, T., Yokoo, J., Kato, A., Shioda, T., Nagai, Y., Gotoh, B., 2001. Sendai virus C protein physically associates with Stat1. Genes Cells 6, 545–557.
Taniguchi, T., Takaoka, A., 2002. The interferon-α/β system in antiviral responses: a multimodal machinery of gene regulation by the IRF family of transcription factors. Curr. Opin. Immunol. 14, 111–116.
Tashiro, M., Homma, M., 1983. Pneumotropism of Sendai virus in relation to protease-mediated activation in mouse lung. Infect. Immun. 39, 879–888.
Yoneyama, M., Kikuchi, M., Natsukawa, T., Shinobu, N., Imaizumi, T., Miyagishi, M., Taira, K., Akira, S., Fujita, T., 2004. The RNA helicase RIG-I has essential function in double-stranded RNA-induced innate antiviral responses. Nat. Immunol. 5, 730–737.
Yoneyama, M., Kikuchi, M., Matsumoto, K., Imaizumi, T., Miyagishi, M., Taira, K., Foy, E., Loo, Y.M., Gale Jr., M., Akira, S., Yonehara, S., Kato, A., Fujita, T., 2005. Shared and unique functions of the DExD/H-box helicases RIG-I, MDA5, and LGP2 in antiviral innate immunity. J. Immunol. 175, 2851–2858.